Shepard.

A Study Of The Amino Acids Of Barley.

A STUDY OF THE AMINO ACIDS OF BARLEY

BY

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THESIS

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Albert Durand Shepard

ENTITLED A Study of the Amino Acids of Barley

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science in Chemistry

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Committee on Final Examination*

*Required for doctor's degree but not for master's.

A STUDY OF THE AMINO ACIDS OF BARLEY

Historical

For a long time it has been understood in a vague way that the chemical differences in proteins as determined by their amino acid content must be of considerable significance in metabolic processes.* During the processes of digestion the protein molecules are hydrolysed and split up into amino acids and other products before absorption takes place in the intestines.

*The Science of Nutrition by Graham Lusk, PhD. Sc.D. F. R. S. Second Edition Page 113.

*The Amino acids are then absorbed unchanged through the walls of the intestines passing into the blood stream, which conducts them to the various tissues of the body.

*Jour. of Biol. Chem. Vol. 17 Page 325 1914.

The amino acids are then absorbed by the tissues without undergoing any immediate chemical change. *The absorption although rapid is never complete, the blood always containing small amounts of amino acids.

*Journal Biol. Chem. Vol. 12 410 1912..

Each tissue absorbs the amino acid or acids which it needs. The ways in which the free amino acids are utilized, according to *Van Slyke and Meyer, are as follows:

I. The amino acids serve as a reserve energy supply

II The amino acids are merely intermediate steps in the construction and breakdown of the tissue proteins.

The views of Van Slyke and Meyers may be sumarized as follows:

"The amino acids appear, therefore to be intermediate steps, not only in the synthesis, but in the breaking down of body proteins."

"The failure to increase the free amino acid content of the tissues by high protein feeding indicates, furthermore, that when nitrogen is retained in the organism it is not to an appreciable extent, as stored digestion products, but rather as body protein."*

*Journal of Biol. Chem. 16 P. 213-231

The steps in the metabolism of the amino acids are not known, but all of the tissues during metabolism form urea which probably with carbon dioxide and water form the end products.*

*Journal Biol. Chem. Vol. 12 P. 161

Since the amino acids are the true nitrogenous nutritive agents, it seems reasonable to expect that they would play a more specific role in the processes of nutrition than a mere carrier of nitrogen. *The proteins can best be compared by their amino acid content. As the ingested proteins are equivalent in their nutritive efficacy for maintenance and growth, to the amino acids which they yield.

*Journal of Biol. Chem. Vol. 17. 331 1914.

A comparison of the results obtained in feeding experiments where pure proteins were fed, based on the amino acid content of the proteins has given us the following *facts:

*Osborne & Mendel Journal Biol. Chem. 17 325. 1914.

I In the absence of the single amino acid tryptaphane nitrogen equilibrium can not be attained.

II The animal body can not synthesize tryptaphane.

III Lysine is indispensable for the functions of growth.

IV The animal body can not synthesize lysine.

The problems of nutrition are concerned with other factors than a sufficiency of nitrogen. Gelatine contains very nearly the same quantity of nitrogen as protein; it breaks up on chemical treatment into the same amino-acids, except that it does not yield tyrasine cystine, and tryptaphane. It will not maintain nitrogen equilibrium; it lacks the amino acid necessary for maintenance. A study of the amino acid content gives us a more nearly correct basis as to the true value of a food than could be ascertained from the protein content. Some proteins lack the amino-acids necessary for maintenance and growth; yet they contain as much nitrogen as proteins which supply these acids. Thus the total amount of nitrogen can not serve as a reliable nutritive index, for the nitrogen balance can be maintained with a smaller amount of total nitrogen than does exist in some of the proteins that are in themselves inadequate for maintenance.

The qualitaive amino-acid content of most of the isolated proteins has been determined.*

*Journal Biol. Chem. 10 43-55 (1911)

But no accurate scheme of analysis has been described for the quantitative separation of the mixed proteins as they occur in nature. It is impossible to calculate the amino-acid content from a knowledge of the total protein, so it is necessary to study

the hydrolytic products, the amino acids, to obtain this information.

The quantitative separation and estimation of the amino acids has been a matter of considerable difficulty. The estimation of the amino acid is a simple matter, but the quantitative separation has been a difficult problem. The following methods have been employed for their separation but these methods do not all yield quantitative results.

I Fractional crystallization.

II Fractional precipitation, using a reagent which precipitates only one or two of the acids present in the mixture. (Phosphotungstic acid precipitates arginine, lysine, hystidine and tryptaphane)

III Fractional distillation of the esters, the compounds being converted into their ethyl esters, which are dried and distilled under low pressure. Since they have different boiling points, they can be separated.

IV Determination of the chemical groups characteristic of the different amino-acids; e.g., cystine is estimated by its sulfur content.

The fractional crystallization method was introduced in 1901 by Emil Fischer. Fischer applied this method to the mono-amino acids. His results are not strictly quantitative, as about 30% of the products are unaccounted for. In "The Chemical Constitution of The Proteins," Part I by R. H. Aders Plimmer, is given two methods for the determination of the amino acids in a hydrolysis mixture. Plimmer divides these acids into mono-amino

and diamino groups and applies a different method to each. The monoamino acids resulting from 250-500 grams of protein, after the removal of the diamino acids with Phospho tungstic acid, are separated by the esterification method. The method does not give quantitative results.

The diamino acids are determined in the hydrolysis mixture from 25-50 of protein, after their precipitation with phospho tungstic acid, by the formation of their silver salts. These salts have different degrees of solubility and can be separated quantitatively. As the separation of the two groups of acids is not quantitative, they can not be determined in the same sample.

It is obvious that the two methods described by Plimmer would not be practical in the analysis of feeding-stuffs:

I The results obtained are not quantitative.

II The amount of feeding stuff necessary to yield 250-500 grams of protein is very large for ordinary laboratory manipulation.

III The two methods can not be run satisfactorily on the same hydrolysis mixture.

The Van Slyke *method followed in the analysis of barley

*Journal Biol. Chem. Vol. 10 18-55 1911.

was used by him in determining the amino-acid content of single and mixed samples of pure protein. His method is based on the reactions of the chemical groups characteristic of the amino acids. In his method he also makes use of the fractional precipitation, for the removal of cystine arginine, lysine, and histidine. This method gave very good results when used with pure pro-

teins; *less than 4% of the nitrogen being unaccounted for in any case and less than 1% in most cases.

*Journal of Biol. Chem. Vol. 10 43-55 1911.

At the present time this is the only method that has been used for the determination of the amino acids of feeding stuffs, and it has these advantages over any other method yet described for the determination of amino-acids:

I The mon amino and di amino nitrogen is determined in the same hydrolysis mixture.

II The results obtained are quantitative.

III The method does not require any high degree of skill in manipulations.

IV The sample required for the determination is not large. (6-8 grams of protein)

Experimental

The object of this investigation was to determine if possible the amino-acid content of barley, since at that time no data had been published on the subject and since the "current trend of the investigation of the chemistry of nutrition is emphasizing the significance of the amino-acids as the fundamental factors in all problems in which hitherto the role of proteins has been involved".*

*Journal Biol. Chem. Vol.. 17 Page 325 (1914)

As the amino acids have different nutritive values, a comparison of the amino-acid content of barley with that of other feedingstuffs would give a means of determining its relative value as a food. A knowledge of the amino-acid content would also be of value in interpeting the results of past feeding experiments and of experiments to be carried on in the future.

In this work with barley attempts were made to determine quantitatively, by the Van Slyke method, the amino acids resulting from an acid hydrolysis. The determinations possibly by his method are ammonia, Humin (melanine) Arginine, Cystine, Histidine, Lysine, amono and nonamino nitrogen in the filtrate from the bases. Tryptaphane, the amono acid so necessary to life is destroyed during acid hydrolysis* in the presence of oxygen carries

*Journal Biol. Chem. Vol. 22 P. 369-389 (1915)

and can not be determined by this method.

With the exception of changes in volume and the amounts of the original sample the method as given in the Journal of Biologi-

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cal Chemistry Volume 10 P. 15-43 (1911) was followed in detail. A very brief review of the method with such modifications as were made is given below.

Method used in the Analysis

HYDROLYSIS. About 25 grams of barley were heated in 250 c.c of 20% HCl on the water both for four hours and then bailed for 22 hours. The hydrolysis was then stopped and a 2 c.c. sample was taken. This was diluted with 1 c.c. of nitrogen-free water and the amino nitrogen determined with the Van Slyke apparatus. This was repeated at the intervals of 4 hours until the volume became constant. This required 26 hours. Before and after each sampling the flask and contents were weighed in order that a correction could be applied in case a concentration in volume occurred.

AMMONIA. After concentrating the hydrolyzed protein solution under diminished pressure it was diluted to 250 c.c. with water; 10 c.c. samples were withdrawn and used for Kjeldahl analysis which gave the total nitrogen.

100 c.c. samples were then taken and, with the apparatus shown in Journal Biological Chemistry Vol. 10, P. 21 (1911), the ammonia was determined. The sample was first treated with alcohol to prevent foaming and then made alkaline with calcium hydroxide. The solution was then distilled under reduced pressure. The ammonia was collected in standard acid alizarine sulfinate being used as an indicator. The excess acid was then titrated with sodium hydroxide.

MELANINE. The solution left in the flask after the de-

termination of ammonia was filtered and the precipitate washed free from chlorides with ammonia-free water. The residue with the paper was then submitted to Kjeldahl analysis 40-60 c.c. of sulfuric acid being required for the digestion.

(The Bases, Cystine, Lysine, Arginine and Histidine). - The filtrate from the melanine was neutralized with hydröchloric acid, and then concentrated to about 50 c.c. in vacuum. It was then transferred to a 250 c.c. flask, 18 c.c. of concentrated hydrochloric acid was added and a solution containing 15 grams of phospho tungstic acid was used to precipitate the bases. The solution was then diluted to the mark with water, and heated on the water bath until the precipitated bases were nearly redissolved. The bases reprecipitated on cooling. After 48 hours the bases were filtered on a Buechner fitted through a hardened paper. The washing was carried on with a solution containing 2.5% of phospho tungstic acid and 3.5% hydrochloric. After the addition of 10 c.c. of washing solution the bases were stirred until a fine suspension was obtained, and then sucked dry. This was repeated until the filtrate came free from calcium.

The precipitated bases were then transferred to a liter flask, the small amounts left on the filter paper being dissolved in water made alkaline with sodium hydroxide. A few drops of phenalphtalein were then added and the solution made alkaline with 50% NaOH solution. Not more than two drops of alkali were added in excess.

The solution was diluted with water to about 800 c.c. and the phospho tungstic acid precipitated with a slight excess of

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[illegible]

3-% barium chloride.

The precipitate of barium phospho tungstate was then filtered and washed free from chlorides. The filtrate was concentrated in vacuo to about 50 c.c. A precipitate of barium phospho tungstate separated and the solution was again filtered and washed, the filtrate being concentrated in vacuo to about 50 c.c. and transferred to a 100 c.c measuring flask.

ARGININE. The determination of arginine is based on the fact first noted by Osborne, Leavenworth, and Brautlecht, that arginine, when boiled with dilute alkali evolves one-half its nitrogen in the form of ammonia. The explanation of the reaction is that, as shown by Schulze and Wintustein, arginine when heated with alkaline solutions decomposes into one molecule each of ornithine and urea. The urea then hydrolizes into ammonia under the conditions described below the reaction is quantitative.

Of the 100 c.c. of solution containing the bases, 25 c.c. are placed in a 200 c.c round bottom Jena flask of the apparatus shown in Fig. 2, Journal Biol. Chem. Vol. 10, 26 (1911). The Folin bulbs were connected at the top of the condenser by means of a rubber stopper. In the Folin bulb was placed 10 c.c. of standard acid, with alizarin sulfenate as an indicator. To the solution in the flask were added 12.5 grams of solid KOH, and several bits of porcelain, glass beads, and pumice. The solution was then boiled gently for exactly six hours.

The boiling is the stopped and the condenser rinsed into the flask with 100 c.c. of water. The flask was then connected

with the black tin condenser and 100 c.c. were distilled into the original 10 c.c. of standard acid. The excess of acid was then titrated back as usual.

Each cubic centimeter of N/10 acid neutralized by the ammonia indicates 0.0028 gram of arginine nitrogen in the solution decomposed, 000.056 grams in the total solution of the bases. If cystine is present 18% of its nitrogen is evolved as ammonia and a correctionto the arginine figure must be made. This correction is negligable with proteins other than the keratins

TOTAL NITROGEN OF THE BASES. - The solution used for the determination of the arginine was transferred to a Kjildahl flask of 600 c.c. capacity. Concentrated sulfuric acid was then added and the solution digested as in the ordinary Kjildahl method. When the digestion was complete the solution was made alkaline and distilled. Each cubic centimeter of N/10 acid neutralized is added to the acid neutralized in the arginine determination and the sum multiplied by 0.0028. This gives the total nitrogen content of the bases.

CYSTINE. - The amount of cystine is determined by the sulfur content. 20 c.c. of the solution containing the bases were acidified with hydrochloric acid and after the addition of 10 c.c. of Denis solution was evaporated to dryness on a water bath. The mixture was then gradually heated to redness and maintained at that temperature for fifteen minutes. The residue was then taken up in hydrochloric acid and water, heated to boiling, and the sulfate precipitated with barium chloride. After standing an hour, it was filtered, and washed, ignited in a tared crucible and weighed. Each milligram of barium sulfate indicates

.06 mg. of cystine nitrogen in the portion of solution analysed, or .3 mg. in the total solution of the bases.*

TOTAL NITROGEN IN THE FILTRATE FROM THE BASES. - The combined filtrate and washings from the precipitate of the bases was made neutral with sodium hydroxide and acetic acid, concentrated in vacuo, and then made up to a volume of 150 c.c. Duplicate portions of 25 c.c. each were taken for Kjeldahl analysis.

NITROGEN IN THE FILTRATE FROM THE BASES. - 10 c.c. portions of the filtrate were used for the amino determinations. These were run for ten minutes to insure complete reaction with the nitrous acid.

*Calculation of Histidine and Lysine. See the Journal of Biol. Chem. Vol. 10 p. 29-30 (1911)

Analytical Data and Results

The analytical results and data are given in full on the following pages. After hydrolysis the solution was made up to 250 c.c.

Total nitrogen was determined on aliquot portions of this solution. 100 c.c. portions were used for the determination of ammonia and melanine nitrogen and for the precipitation of the bases. The solution of the bases was made up to 50 c.c. and arginine, cystine, amino and total nitrogen were determined on aliquot portions. The filtrate from the bases was made to 150 c.c. amino and total nitrogen were determined on aliquot portions.

The results are all expressed in terms of 100 c.c. of the hydrolyzed solution for total nitrogen. The results expressed in percentage of the feeding stuff are figured from the weight of the feeding stuff in 100 c.c. of the hydrolyzed solution.

A volume factor is given which relates to the 100 c.c. portion of hydrolyzed solution.

Table I. Total Nitrogen In The Hydrolyzed Solution.

Sample	Volume Standard acid taken	N of 1cc. of acid	1cc. of Alkali in terms of acid	Reagent factor	Volume of Strong Alkali required	Volume Factor	Wt. of total N in 10cc. of Hydrolyzed Solution	Per cent of N in the feeding stuff	Wt. of feeding stuff corresponds to mt. of total N in 100 cc. solution
A	15	.00258	0.449	0.159	13.00	25	0.580750	2.368	24.525
A	15	.00258	0.449	0.159	12.10	25	0.606825	2.368	25.626
A	15	.00258	0.449	0.159	12.65	25	0.590875	2.368	24.952
B	15	.00258	0.449	0.159	12.10	25	0.606825	2.368	25.626
B	15	.00258	0.449	0.159	11.85	25	0.614050	2.368	25.931
B	15	.00258	0.449	0.159	11.90	25	0.612625	2.368	25.871
C	15	.00258	0.444	0.130	13.62	25	0.569075	2.368	24.032
C	15	.00258	0.444	0.130	13.52	25	0.571925	2.368	24.152
C	15	.00258	0.444	0.130	13.45	25	0.573925	2.368	24.237
D	15	.00258	0.444	0.130	13.40	25	0.575350	2.368	24.297
D	15	.00258	0.444	0.130	13.25	25	0.579650	2.368	24.478
D	15	.00258	0.444	0.130	13.27	25	0.579075	2.368	24.454

Table No. II

Amid Nitrogen In The Hydrolyzed Solution

Sample	Volume of sample taken	Volume of strong acid taken	N of 1cc. of acid	1cc. of alkali in terms of acid	Volume of strong alkali required	Wt. of ammonia N in sample	Amid nitrogen as per cent of total N	Amid nitrogen as percentage of feeding stuff
A	100	20	0.00258	0.449	11.40	0.038393	16.191	0.383
B	100	20	0.00258	0.449	11.80	0.037931	15.516	0.367
C	100	20	0.00258	0.444	14.40	0.035103	15.352	0.364
D	100	20	0.00258	0.444	13.80	0.035792	15.480	0.367

Table No. III

Melanine Nitrogen In The Hydrolyzed Solution

Sample	Vol. of sample taken	Vol. of strong acid taken	N of 1cc. of acid	1cc. of alkali in terms of acid	Reagent factor	Vol. of Alkali required	Wt. of Melanine nitrogen	Melanine N as percentage of total N	Melanine N as percentage of feeding stuff
A	100	15	.00258	0.449	.405	9.70	0.026419	11.141	0.264
B	100	15	.00258	0.449	.405	12.95?	0.022652	0.266	0.219
C	100	15	.00258	0.444	.405	12.35	0.023509	10.281	0.243
D	100	15	.00258	0.444	.405	11.85	0.024082	10.416	0.247

Table No. IV

Arginine Nitrogen In The Hydrolyzed Solution

Sample	Vol. of strong acid taken	N of 1cc. of acid	1cc. of alkali in terms of acid	Volume of strong alkali required	Vol. factor	Corrected Wt. of total arginine N in solution of bases	Arginine N as per cent of total N	Arginine N as percentage of feeding stuff
A	10	0.00258	.444	17.65	4	0.025524	10.764	0.255
B	10	0.00258	.444	16.5	4	0.030796	12.597	0.298
C	10	0.00258	.444	30.80	4	0.016876	7.380	0.175
D	10	0.00258	.444	30.15	4	0.019848	9.584	9.203

Table No. V

Total Nitrogen of the Bases In The Hydrolyzed Solution

Sample	Vol. of strong acid taken	N of 1cc. of acid	1cc. of alkali in terms of acid	Reagent factor	Vol. of strong alkali required	Vol. factor	Uncorrected wt. of one half of the arginine N in total solution of bases	Uncorrected wt. of total N of bases in 10cc. of hydrolyzed solution
A	15	.00258	.444	.130	22.15	2	.011662	.037142
A	20	.00258	.444	.139	38.28	2	.010892	.025676
B	15	.00258	.444	.130	22.10	2	.013798	.039898
B	Lost	.00258	.444	Lost	Lost	Lost	Lost	Lost
C	15	.00258	.444	.139	26.45	2	.006838	.022922
C	10	.00258	.444	.139	12.32	2	.008870	.031528
D	15	.00258	.444	Lost	Lost	Lost	Lost	Lost
D	10	.00258	.444	.139	16.70	2	.009676	.022298

Table No. VI

Cystine Nitrogen In The Hydrolyzed Solution

Sample	Wt. of dish + $BaSO_4$	Wt. of Empty Dish	Wt. of $BaSo_4$	Wt. of $BaSo_4$ from blank	Cystine N as percentage of total nitrgen	Vol. factor	Corrected wt. of cystine N in total solution of bases	Cystine N as percentage of feeding stuff
A	6.6100	6.6068	.0005	.0026	1.160	5	.002750	.027
B	6.6151	6.6101	.0024	.0026	1.358	5	.003320	.032
C	6.6096	6.6070	.0000	.0026	0.000	5	.000000	.000
D	6.6160	6.6097	.0037	.0026	1.605	5	.003710	.138

Table VII

Animo Nitrogen In The Solution Of The Bases

Sample	Vol. of gas	Temperature	Barometer	Vol. of gas by Black	Vol. factor	Uncorrected Wt. of Animo N in total solution of the bases	Pressure
A	5.55	29.8	29.15	.65	5	.012885	740.4
B	5.50	27.0	29.08	.65	5	.015590	738.6
C	4.40	28.0	28.85	.65	5	.009855	732.8
D	5.20	29.5	28.96	.65	5	.011905	735.6

Table VIII

Calculation of Histidine Nitrogen

Sample	Uncorrected wt.of total N of the bases in 100cc.of the hydrolyzed solution	Uncorrected wt. of Animo N of the bases in 100cc. of hydrolyzed solution	Uncorrected wt. of N in Animo N of the bases in 100cc. of the hydrolyzed solution	Uncorrected wt. of Arginine N in 100cc. of the hydrolyzed solution	Corrected wt. of Histidine N in 100 cc. of hydrolyzed solution	Histidine N as percentage of total N	Histidine N as percentage of feeding stuff
A	.037142	.012885	.024257	.022324	.015071	6.356	.151
B	.039898	.015590	.024308	.027596	.009216	3.770	.089
C	.022922	.009855	.013067	.013676	.008015	3.505	.083
D	....	.011905	....	.016648	....	.....	....

Table IX

Calculation of Lysine Nitrogen

Sample	Uncorrected wt.of total N of the bases in 100cc.of hydrolyzed solution	Uncorrected wt.of Arginine N in 100 cc.of hydrolyzed solution	Uncorrected wt.of lystine N in 100cc. of hydrolyzed solution	Uncorrected wt.of histidine N in 100 cc.of hydrolyzed solution	Corrected wt.of lysine in 100cc.of the hydrolyzed solution	Lysine as percentage of total N	Lysine as percentage of feeding stuff
A	.037142	.022324	.000150	.011271	.003897	1.643	.039
B	.039898	.027596	.000720	.005416	.006666	2.727	.065
C	.002292	.013676	....	.004215	....	.....	....
D	.022298	.016648	....	....	....	.....	....

Table X

Total N In The Filtrate From The Bases

Sample	Volume of strong acid taken	N of 1cc. of acid	1cc. of alkali in terms of acid	Reagent factor	Volume of strong alkali required	Volume factor	Uncorrected wt.of N of "filtrate" in 100cc.of the hydrolyzed solution
A	15	.00258	.444	.130	25.25	15	.141600
A	15	.00258	.444	.130	24.70	15	.151050
A	15	.00258	.444	.130	25.20	15	.142455
A	15	.00258	.444	.130	13.25	6	.139116
A	15	.00258	.444	.130	13.36	6	.138360
B	15	.00258	.444	.130	25.25	15	.141600
B	15	.00258	.444	.130	25.60	15	.135600
B	15	.00258	.444	.130	25.45	15	.138165
B	15	.00258	.444	.130	13.60	6	.136722
B	15	.00258	.444	.130	13.70	6	.136020
C	15.1	.00258	.444	.130	25.75	15	.136875
C	15	.00258	.444	.130	25.65	15	.138585
C	15	.00258	.444	.130	25.35	15	.143775
D	15	.00258	.444	.130	25.38	15	.139365
D	15	.00258	.444	.130	25.40	15	.139005
D	15	.00258	.444	.130	25.48	15	.137655

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Table XI

Amino Nitrogen In The Filtrate From The Bases

Sample	Volume of gas	Barometer	Temperature	Vol. of gas in blank	Volume factor	Corrected wt. of Amino N of filtrate from 100 cc. of hydrolyzed solution	Amino N as percentage of total nitrogen	Amino N as percentage of feeding stuff	Pressure
A	14.88	28.841	27.5	.4	15	0.237127	46.072	1.078	732.5
A	14.88	28.841	27.5	.4	15	0.237127	46.072	1.078	732.5
B	15.10	28.841	27.5	.4	15	0.244467	45.395	1.075	732.5
B	15.02	28.841	27.0	.4	15	0.244467	45.272	1.072	732.5
C	13.92	29.190	27.0	.4	15	0.228657	45.175	1.070	741.4
C	13.91	29.190	27.0	.4	15	0.228657	45.142	1.069	741.4
D	14.60	28.850	28.5	.4	15	0.231210	46.032	1.090	732.8
D	14.55	28.850	28.5	.4	15	0.231210	45.863	1.086	732.8

Table XII

Non Amino Nitrogen In The Filtrate From the Bases

Sample	Uncorrected wt. of N of the "filtrate"	Uncorrected wt. of Amino nitrogen of the "filtrate"	Corrected Wt. of Non Amino Nitrogen in the "filtrate	Non Amino N of "filtrate" as per cent of total N	Non Amino N of "filtrate" as percent of feeding stuff
A	0.142028	0.109250	0.027878	11.757	0.278
B	0.139883	0.110825	0.024158	9.882	0.234
C	0.139745	0.103258	0.031587	13.814	0.327
D	0.139183	0.106235	0.028048	12.131	0.287

Table XIII

Results Expressed in Percentage of the Total Nitrogen of the Feedingstuff

Ammonia N.	Humin N.	Arginine N.	Cystine N.	Histina N.	Lysine N.	Amino N in filtrate from bases	Non amino N in filtrate from bases	Total N by summation
16.191	11.141	10.764	1.160	6.356	1.643	46.072	11.757	
15.516	9.266	12.597	1.358	3.770	2.727	46.072	9.882	
15.352	10.281	7.380	1.605	3.505		45.395	14.814	
15.480	10.416	8.584				45.272		
						45.175		
						45.142		
						45.032		
						45.863		
Average -								
15.634	10.276	9.831	1.374	4.544	2.185	45.654	11.896	101.394

Table XIV

Results Expressed in Percentage of Feedingstuff

Ammonia N.	Humin N.	Argi-nine N.	Cys-tine N.	His-tidine N.	Lys-ine N.	Amino N in fil-trate from bases	Non amino N in fil-trate from bases	Total N by summa-tion	Total N by Analysis
.383	.264	.255	.027	.151	.039	1.078	.278		
.367	.219	.298	.032	.089	.065	1.078	.234		
.364	.243	.175	.038	.083		1.075	.327		
.367	.247	.203				1.072	.287		
						1.070			
						1.069			
						1.090			
						1.086			
Average -									
.370	.243	.233	.032	.107	.052	1.077	.281	2.395	2.368

Discussion of Results

The melanine-nitrogen content of barley when compared with that for pure isolated proteins, as reported by Van Slyke,* is

*Journal Biol. Chem. 10 54 (1911)

seen to be unusually high. The highest result reported by Van Slyke was 3.6% in the case of ox hemoglobin. The melanine content of barley is 3.4 times the highese results reported by Van Slyke. It is more than probable that the variation is due in part to incomplete hydrolysis of the proteins occluded in the woody fiber of the grain. *Annie Homer has demonstrated that,

*Journal of Biological Chemistry Vol. 22 P. 369-389 (1915)

during acid hydrolysis, any oxygen carrier will cause the destruction of tryptophane with the formation of melanines. Hunin contains in addition to the nitrogen from tryptaphane, the nitrogen from other absorbed amino acids. Experiments have shown that under the conditions of hydrolysis Lysine and *Cystine evolve 4.7 and 6.3% of their respective nitrogen, as humin bodies.

*Journal of the American Chemical Society 37 - 2768 1915

The ammonia content of hydrolyzed proteins has become especially significant since Osborne, Leavenworth, and Brautlecht have shown that the nitrogen of the ammonia is usually equal to that of the dicarbolylic acid with which it is combined, in the protein molecule, in the form of acid-amid radicles. In 1915, E. H. Nollan published some results* on the amino-acid content of

*Journal Biol. Chem. Vol. 21-611 1915.

certain commercial feedingstuffs. His results on barley do not agree with the results reported in this paper. His ammonia nitrogen is about 1% higher. His melanine nitrogen is 6% lower and the cystine nitrogen is 3% higher. His arginine is 1% lower. His histidine nitrogen is 3% higher. Lysine was reported absent. The differences in the results in the case of melanine is probably due to the fact that after the hydrolysis the unhydrolyzed portion was filtered off by him. This would cause low results, as the unhydrolyzed portion contains absorbed amino-acids and melanine-like bodies. The treatment of the sample, previous to hydrolysis, may be in part responsible for some of the differences in results. In his work the fat-like bodies were extracted. This would prevent the formation of glycerol, which according to Maillard would interfere with the hydrolysis. As the nitrogen in the unhydrolyzed portion was not determined he could not calculate the amino acids in percent of the feedingstuff.

In barley the mon amino-acid nitrogen constitutes over one-half the total nitrogen of the feeding stuff. This would indicate that barley is especially rich in tyrasine which is a normal constituent of hardien. Since the role played by lysine in nutrition is for the function of growth it is of interest to note the low lysine figure. Next to cystine this result is the lowest given and is perhaps of the most importance. For the function of growth barley can never be of value but when fed with other feedingstuffs rich in lysine it serves for the purpose of maintenance.

The sulfur in the body is believed to be derived exclu-

sively from the breaking down of proteins.* The content of

*Lusk. The Science of Nutrition. page 67.

cystine, which has sulfur in its molecule, is high, showing that the combined sulfur, furnished by the amino acid in the body can be supplied from barley. It is evident from the results obtained that the nitrogen of the feedingstuff is distributed widely amongst the various amino-acids. For the purpose of comparison are given the results of several feeding-stuffs arranged according to their increasing content of the different forms of nitrogen determined by the Van Slyke method and expressed in percentage of the total nitrogen of the feeding stuff;

Ammonia N.		Melanine N.		Arginine N.	
Blood meal	5.85	Blood meal	3.85	Alfalfa hay ...	7.68
Tankage	6.58	Tankage	4.40	Whole wheat ...	7.99
Alfalfa hay ...	8.44	White soy beans	6.63	Rolled wheat ..	8.20
White soy beans	10.12	Cottonseed meal	7.78	Blood meal	9.16
Cottonseed meal	10.45	Rolled wheat ..	9.04	Barley	9.83
Oats	13.06	Whole wheat ...	9.21	Oats	11.42
Barley	15.63	Oats	9.94	White soy beans	12.67
Rolled wheat ..	17.04	Barley	10.28	Tankage	14.15
Whole wheat ...	17.59	Alfalfa hay ...	15.79	Cottonseed meal	19.52

Histidine N.		Cystine N.		Lysine N.	
Whole wheat	1.67	Cottonseed meal	0.65	Barley	2.19
Rolled wheat ...	3.21	White soy beans	0.67	Whole wheat	2.47
Oats	4.32	Blood meal	0.69	Rolled wheat ...	2.48
Barley	4.54	Alfalfa hay	0.88	Oats	3.49
Tankage	4.94	Oats	1.16	Alfalfa hay	4.10
Cottonseed meal	5.47	Tankage	1.28	Cottonseed meal	4.78
White soy beans	5.77	Whole wheat	1.34	White soy beans	6.14
Alfalfa hay	7.44	Barley	1.37	Tankage	7.48
Blood meal	8.53	Rolled wheat ...	1.66	Blood meal	9.73

Amino N in the filtrate from the bases		Non-amino-N in the filtrate from the bases	
Cottonseed meal	42.82	Blood meal	4.42
Alfalfa hay ...	44.02	Cottonseed meal	5.43
Barley	45.65	Tankage	7.27
Whole wheat ...	47.67	Oats	7.90
Rolled wheat ..	47.70	White soy beans	8.56
White soy beans	49.79	Alfalfa hay ...	9.79
Oats	51.72	Barley	11.90
Tankage	52.39	Whole wheat ...	13.59
Blood meal	56.57	Rolled wheat ..	13.95

Diamino acid N.		Monoamino Acid N.	
Whole wheat ...	13.47	Cottonseed meal	48.25
Rolled wheat ..	15.54	Alfalfa hay ...	54.81
Barley	16.55	White soy beans	58.35
Alfalfa hay ...	20.10	Oats	59.62
Oats	20.39	Tankage	59.66
White soy beans	25.25	Barley	59.69
Tankage	27.88	Blood meal	60.99
Blood meal	28.11	Whole wheat ...	61.26
Cottonseed meal	30.32	Rolled wheat ..	61.65

An examination of the results given in the table shows many individual peculiarities of the mixed proteins. Among such peculiarities may be mentioned the low value for lysine nitrogen in barley compared with the high content of blood meal. The manner in which barley differs from packing house products is noteworthy. The ammonia nitrogen of blood meal is very low,about 6%, while barley has nearly three times as much. In the case of melanine nitrogen the ratio is about the same; i.e., 1 : 3. This can be explained by the relative high content of fiber in barley. The amount of arginine nitrogen is about the same for barley and blood meal. In the case of histidine nitrogen blood meal has nearly three times as much as barley; while, barley has twice as much cystine nitrogen as blood meal. The greatest variation is seen in the lysine content, blood meal having nearly five times as much lysine nitrogen as barley.

The different forms of nitrogen in barley compare very favorably with those of other grains. In ammonia nitrogen barley has more than oats and less than wheat. There is only about 1% difference in the melanine content of the three grains, barley containing the highest value. The arginine value is greatest for oats, but barley contains more than wheat. In cystine the wheat and barley have almost the same figure, the oats yielding the lowest result. For the lysine content, oats yield the highest figure, showing that for the functions of growth, the best results can be obtained by feeding oats and wheat. Alfalfa hay has nearly twice the amount of lysine nitrogen that is present in barley. Alfalfa hay contains more melanine, histidine, and diamino-acid nitrogen than barley but is low in arginine, ammonia, and monamino nitrogen.

Summary

The science of nutrition is emphasizing the value of the amino-acids and more emphasis will be placed on their content in the natural proteins of feedingstuffs when the specific role of a greater number is determined. Feeding experiments can be conducted having a balanced ration figured in terms of amino acid in place of protein. In practical feeding food stuffs which are lacking in any of the necessary forms of nitrogen can be supplied by mixing with those having a high content of that particular form of nitrogen. It is evident from the results obtained that the Van Slyke method, for the determination of the chemical groups, characteristic of the amino acids can be applied directly to the quantitative determination of the amino acids feeding-stuffs. The following facts are also evident:

I The results reported on barley do not with the results reported by Nollan, probably because of the differences in the details of the method.

II The presence of carbohydrates, oxygen carriers, and fiber are responsible for the high results for humin nitrogen, and are a source of error in the determination.

III There are wide variations in the amino-acid content of the various feedingstuffs.

IV Barley is deficient in lysine nitrogen and will not serve well for the functions of growth.

V The results from these and other similar investigations will be helpful in the interpretation of past and future feeding experiments.